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Chemical Composition, Antibacterial and Antioxidant Activities of Essential oil from *Leonurus pseudomacranthus* Kitag

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Abstract: This study investigated the chemical composition and in vitro antibacterial and antioxidant activities of the essential oil obtained by hydrodistillation from the aerial parts of *Leonurus pseudomacranthus* Kitag for the first time. The chemical composition was studied by GC-FID and GC-MS. Forty-nine compounds accounting for 91.1% of the essential oil were identified. The major components were sclareol (34.8%), β-caryophyllene (7.1%), precocene (I) (6.3%) and α-muurolene (5.3%). The antibacterial activity of the essential oil was assessed by the disc diffusion and microdilution methods. The essential oil showed excellent antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* with MIC values of 0.039 mg/mL and 0.156 mg/mL, respectively. Moreover, the antioxidant potential was evaluated by DPPH, ABTS and FRAP assays. The essential oil gave IC_{50} values of 1.513 mg/mL, 0.152 mg/mL in DPPH and ABTS methods, and a Trolox equivalent concentration of 33.63 μmol Trolox × g^{-1} in FRAP method. The results indicated that the essential oil could be regarded as a promising product for pharmaceutical and food industry after more detailed study.

Keywords: *Leonurus pseudomacranthus* Kitag; essential oil; antibacterial activity; antioxidant activity.

1. Plant Source

The aerial parts of *Leonurus pseudomacranthus* Kitag were collected from western hills of Meizhou in Guangdong Province of China, during July 2016. The plant material was identified by Associate Prof. Hong Zhao of Marine College, Shandong University. The voucher specimen (No.10436) has been deposited at the Laboratory of Botany of Marine College, Shandong University.

2. Previous Studies

Leonurus pseudomacranthus Kitag, belonging to the genus *Leonurus* in the Labiatae family, is a perennial herb and is mainly distributed in the southern part of China [1]. The aerial parts of *L. pseudomacranthus* have been used for the treatment of menstrual disorders and kidney and urethra problems in traditional Chinese medicine [2]. Several species of the *Leonurus* genus have been reported to possess medicinal properties such as antibacterial [3], anti-gynecological disorder, cardiovascular protection, neuroprotection, anti-inflammation and immunomodulation properties [4]. To the best of our

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knowledge, the chemical composition and biological activity of the essential oil obtained from *Leonurus pseudomacranthus* Kitag have not been investigated. Therefore, we report here the composition of the essential oil obtained from the aerial parts of *L. pseudomacranthus* and its antibacterial and antioxidant activities.

3. Present Study

The aerial parts of the fresh plant material (500g) were hydrodistilled for four hours using a Clevenger apparatus to extract the essential oil in a yield of 0.106% (w/w) of the fresh weight. Identification of the essential oil constituents was performed by comparing GC-MS retention data with retention indices obtained by the combination of the essential oil with n-alkanes as internal standards. (See Supporting Information for the details of the experiment.)

Table 1. Chemical composition of essential oil of *L. pseudomacranthus* Kitag

Compound [a]	RI [b]	RI [c]	% Area[d]	Ref.	Compound [a]	RI [b]	RI [c]	% Area[d]	Ref.
δ-EIemene	1338	1338	0.4	[5]	Cubenol	1644	1644	1.1	[5, 6]
α-Longipinene	1348	1348	2.1	[5,6]	*τ*-Cadinol	1646	1640	1.2	[5]
α-Cubeben	1359	1360	0.5	[5, 6]	*τ*-Muurolol	1652	1648	0.3	[5]
Dehydro-ar-ionene	1367	1359	0.4	[5]	*α*-Cadinol	1664	1663	1.1	[5]
Copaene	1389	1390	0.6	[5, 6]	*Iso*-Longifolol	1712	1712	0.2	[5]
β-Elemene	1401	1399	1.3	[5, 6]	*γ*-Costol	1747	1752	0.6	[5]
β-Caryophyllene	1437	1437	7.1	[5, 6]	Santalcamphor	1764	1774	4.0	[5]
Calarene	1445	1442	0.3	[5]	*cis*-Valerenyl acetate	1813	1805	0.3	[5]
Allo-Aromadendren	1458	1458	1.1	[5, 6]	*trans*-Valerenyl acetate	1823	1832	0.2	[5]
Pricocene I	1474	1472	6.3	[5]	Neophytadiene	1832	1837	1.4	[5]
γ-Muurolene	1480	1480	1.1	[5, 6]	Hexahydrofarnesyl acetone	1839	1837	0.6	[5]
Valencen	1489	1488	0.9	[5, 6]	Diisobutyl phthalate	1868	1868	0.3	[5]
Ledene	1495	1495	2.5	[5]	Sclareol oxide	1916	1906	0.3	[5]
α-Muurolene	1507	1507	5.3	[5, 6]	Pimaradiene	1921	1919	0.2	[5]
δ-Cadinene	1536	1539	1.9	[5, 6]	Cembrene	1944	1941	0.3	[5]
Spathulenol	1553	1553	0.4	[5]	Dibutyl phthalate	1961	1960	0.1	[5]
β-Calacorene	1561	1561	0.5	[5, 6]	*m*-Camphorene	1965	1960	0.3	[5]
Caryophyllene oxide	1574	1574	0.4	[5, 6]	Manoyl oxide	1993	1994	0.4	[5, 6]
Epiglobulol	1581	1582	0.3	[5]	16-Kaurene	2068	2061	0.4	[5]
Guaiol	1592	1593	0.5	[5, 6]	Methyl linolenate	2097	2098	0.1	[5]
Iso-Aromadendrene epoxide	1600	1594	2.4	[5]	Oleic Acid	2139	2140	0.3	[5, 6]
					Sclareol	2212	2220	34.8	[5]
Viridiflorol	1604	1605	1.4	[5, 6]	Larixol	2266	2265	0.3	[5]
Ledol	1615	1616	0.7	[5]	Communic acid	2395	2404	2.7	[5]
trans-Isolongifolanone	1623	1619	0.9	[5]	Diisooctyl phthalate	2542	2540	0.3	[5]
					Sesquiterpene hydrocarbons			19.4	
					Oxygenated sesquiterpenes			13.3	
					Diterpenes hydrocarbons			2.1	
					Oxygenated diterpenes			37.5	
					Total identified			91.1	

[a] are listed in order of their elution from a HP-5MS column; [b] (retention index): RI-non-isothermal Kovats retention indices on a HP-5MS column relative to C_{10}-C_{30} n-alkanes; [c] linear retention indices according to the literature and NIST Chemistry WebBook on a HP-5MS column; [d] The content (%) of the individual components was calculated based on the peak area (FID response)

A total of forty-nine compounds were identified, which represent 91.1% of the total composition of the essential oil. The chemical composition of the essential oil and percentages of components are presented in Table 1. Oxygenated diterpenes were predominant (37.5%), followed by sesquiterpene hydrocarbons (19.4%), oxygenated sesquiterpenes (13.3%) and diterpenes (2.1%). The principal

chemical constituents were found to be sclareol (34.8%), β-caryophyllene (7.1%), precocene (I) (6.3%) and α-muurolene (5.3%). Essential oil compositions of some other *Leonurus* species have been previously studied [3,7,8,9]. In previous studies of the essential oils from *L. cardiaca*, *L. sibiricus*, and *L. japonicus*, β-caryophyllene was identified as the principal compound in each of the species with 39.8%, 35.2%, and 9.9%, respectively [3,7,8]. The presence of β-caryophyllene in a significant amount indicated that the occurrence of β-caryophyllene as a major constituent may be a characteristic of *Leonurus* essential oils. However, the presence of sclareol, precocene (I) and α-muurolene, mentioned in this work as major constituents, had never been previously reported for the *Leonurus* species.

Antibacterial Activity test: The antibacterial activity of the essential oil was estimated by means of disc diffusion [10] and microdilution methods [11,12], and the results are expressed as the inhibition zone diameters (DIZs) and the minimum inhibitory concentrations (MICs) in Table 2. The essential oil of *L.pseudomacranthus* exhibited obvious antibacterial activities against tested Gram-positive bacteria with the DIZ values of (24.9 ± 0.7) and (16.5 ± 0.6) mm for *B. subtilis* and *S. aureus*, respectively. However, this essential oil showed low activity (DIZ: <7.5 mm) towards Gram-negative bacteria. And the MIC values also indicated it had strong antibacterial activity against all selected Gram-positive bacteria. The most susceptible bacterial strain was *Bacillus subtilis* (MIC = 0.039 mg/mL), followed by *Staphylococcus aureus* (MIC = 0.156 mg/mL). However, it did not have significant activity against the Gram-negative bacteria. The probable cause of the susceptibility of Gram-positive bacteria and the relative tolerance of Gram-negative bacteria to essential oils has been correlated with the presence of a hydrophilic outer layer [13]. The outer membrane of Gram-negative bacteria is rich in hydrophilic lipopolysaccharides (LPS) which act as a physical barrier against penetration of hydrophobic components [14]. Generally, the antibacterial properties of essential oils are closely associated with their most abundant components therein [15]. The previous study revealed significant antibacterial activities of sclareol [16] and β-caryophyllene [3]. Synergistic effect between the major and minor components of the essential oil may also contribute to the significant antibacterial activity of the essential oil [15]. Moreover, the effectiveness of the essential oil of *L. pseudomacranthus* against susceptible bacteria was higher than those previously reported for other species of *Leonurus* such as *L. japonicas* [3] and *L. sibiricus* [17].

Table 2. Antibacterial activity of essential oil of *L. pseudomacranthus*

Test strains	[a]Diameter of the inhibition zones (mm)		MIC (mg/mL)	
	Essential Oil	Ch	Essential Oil	Ch
Gram positive				
Bacillus subtilis ATCC 6633	24.9 ± 0.7	28.3±1.0	0.039	0.020
Staphylococcus aureus ATCC 6538	16.5 ± 0.6	25.3±0.8	0.156	0.039
Gram negative				
Escherichia coli ATCC 25922	6.6 ± 0.5	26.2±0.8	>2.50	0.039
Pseudomonas aeruginosa ATCC 27853	6.8 ± 0.7	27.8±0.4	>2.50	0.020

The diameter of the inhibition zones (mm), including the disc diameter (6 mm), are given as the mean ± SD of triplicate experiments. [a]Diameter of the inhibition zones of the essential oil (tested volume, 1 mg/mL×10 μL) ; positive control: Ch, chloramphenicol (tested volume, 0.01 mg/mL)

Antioxidant activity test: The essential oil of *L. pseudomacranthus* was subjected to screening for the possible antioxidant activity by three methods namely DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay [18], ABTS (2,20-azinobis-3-ethylbenzothiazoline-6-sulphonate) radical cation scavenging assay [19] and FRAP (ferric reducing antioxidant potential) assay [20]. The results are presented in Table 3. It was observed that the essential oil of *L. pseudomacranthus* exhibited a weak DPPH radical-scavenging activity with an IC_{50} value of 1.513 mg/mL compared with the standards,

BHT (IC_{50} value of 0.017 mg/mL) and Trolox (IC_{50} value of 0.015 mg/mL). Higher antioxidant activity was detected in the ABTS radical cation scavenging activity assay with an IC_{50} value of 0.152 mg/mL. In view of the results of FRAP assay, the essential oil showed a moderate ferric ion reducing activity (Trolox equivalent antioxidant concentration = 33.63 ± 2.81 μmol Trolox × g^{-1}). Strong antioxidant activity of essential oils has been attributed to their phenolic constituents such as thymol, carvacrol and eugenol [21]. Therefore, the moderate antioxidant activity may be attributed to the low contents of such compounds in the *L. pseudomacranthus* oil.

In summary, the present study indicated that the essential oil obtained from the aerial parts of *L. pseudomacranthus* showed a significant antimicrobial activity against referenced gram-positive strains and also possessed a moderate antioxidant activity. These results showed that the essential oil could be considered as a natural source for isolation of active constituents for food supplements and therapeutic applications. However, further investigation of its activity *in vivo*, is necessary to elaborate and exploit this promise.

Table 3. Results of antioxidant activity in vitro (DPPH, ABTS and FRAP) of essential oil of *L. pseudomacranthus*

Test Sample	DPPH IC_{50} (mg/mL) [a]	ABTS IC_{50} (mg/mL) [a]	FRAP (μmol Trolox × g^{-1})
EO [b]	1.513 ± 0.036	0.152 ± 0.062	33.63 ± 2.81
BHT [c]	0.017 ± 0.001	0.016 ± 0.003	
Trolox [c]	0.015 ± 0.002	0.013 ± 0.005	

[a] IC_{50} = The concentration of compound that affords a 50% reduction in the assay.
[b] EO = Essential oil of *L. pseudomacranthus*
[c] Positive control used.

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Supporting Information

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